AWARD NUMBER: W81XWH-14-1-0420

TITLE: Development of Specific Inhibitors for Breast Cancer-Associated Variants of ErbB2

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CONTRACTING ORGANIZATION: The Research Foundation of State University
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REPORT DATE: October 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2015	Annual	26 Sep 2014 - 25 Sep 2015

4. TITLE AND SUBTITLE

Development of Specific Inhibitors for Breast Cancer-Associated Variants of ErbB2

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0420

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The Research Foundation of SUNY
State University of NY at Stony Brook,
Stony Brook, NY 11794

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The receptor tyrosine kinase HER2/ErbB2 is one of the most highly mutated tyrosine kinases in breast cancer. Several mutations within the ErbB2 kinase domain have been identified in breast cancer patients, but in most cases it is not known whether these mutations increase kinase activity and signaling. The first objective of this project is to provide data linking ErbB2 mutations to their degree of kinase activation. The second objective is to use computational screening methods to identify compounds that can selectively target and inhibit those ErbB2 mutants verified as active. The third objective is to experimentally test a subset of the most promising compounds for the ability to experimentally inhibit specific ErbB2 mutants. The research team consists of two principal investigators at the same institution: Dr. Todd Miller (Initiating PI, Grant Number BC132617) whom has expertise in tyrosine kinase signaling, and Dr. Robert C. Rizzo (Partnering PI, Grant Number, BC132617P1) whom has expertise in computational modeling procedures. This report, prepared by Partnering PI Rizzo and his team, will primarily focus on the computational progress. The Initiating PI Miller has submitted a separate report.

15. SUBJECT TERMS
Tyrosine kinase, ErbB2, inhibitors, virtual ligand screening, kinase assay

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	9	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION:

The receptor tyrosine kinase HER2/ErbB2 is one of the most highly mutated tyrosine kinases in breast cancer. Several mutations within the ErbB2 kinase domain have been identified in breast cancer patients, but in most cases it is not known whether these mutations increase kinase activity and signaling. The first objective of this project is to provide data linking ErbB2 mutations to their degree of kinase activation. The second objective is to use computational screening methods to identify compounds that can selectively target and inhibit those ErbB2 mutants verified as active. The third objective is to experimentally test a subset of the most promising compounds for the ability to experimentally inhibit specific ErbB2 mutants. The research team consists of two principal investigators at the same institution: Dr. Todd Miller (Initiating PI, Grant Number BC132617) whom has expertise in tyrosine kinase signaling, and Dr. Robert C. Rizzo (Partnering PI, Grant Number, BC132617P1) whom has expertise in computational modeling procedures. This report, prepared by Partnering PI Rizzo and his team, will primarily focus on the computational progress. Initiating PI Miller has submitted a separate report.

2. KEYWORDS:

Tyrosine kinase, ErbB2/HER2, enzyme inhibition, protein phosphorylation, computational modeling, virtual screening

3. ACCOMPLISHMENTS:

What are the major goals of the project?

From approved Statement of Work:

Specific Aim 1: Testing cellular activity of ErbB2 mutants. **(Miller laboratory)***	**Timeline**
Major Task 1: Express ErbB2 mutants in NIH3T3 cells	Months 1-4
Major Task 2: Measurements of ErbB2 activity	Months 5-8
Milestone #1: Identification of activated ErbB2 mutants. This will enable work on Specific Aims 2 and 3 to proceed concurrently.	Month 8

Specific Aim 2: Biochemical studies of activated ErbB2 mutants. **(Miller laboratory)***	**Timeline**
Major Task 3: Express and purify ErbB2 mutants	Months 8-12
Major Task 4: In vitro activity measurements	Months 9-15

Specific Aim 3: Identifying inhibitors of ErbB2 mutants. **(Rizzo laboratory)**	**Timeline**
Major Task 5: Produce ErbB2 structures for drug-lead identification	Months 1-12
Milestone #2: Production of computationally-derived pdb files of the structures of activated forms of ErbB2.	Month 12
Major Task 6: Virtual screening and experimental validation	Months 8-24
Milestone #3: Identification of inhibitors for each activated form of ErbB2.	Month 24
Milestone #4: Manuscript on combined use of experimental and computational approaches to identify ErbB2 inhibitors.	Month 24

*** These aims/tasks describe work to be done in Dr. Miller's laboratory, and will not be discussed in detail in this report.**

What was accomplished under these goals?

Figure 1 outlines the overall research strategy of this collaborative project.

The Rizzo lab has made major progress in building and refining both wild type homology models and homology models for three of the four ErbB2 somatic mutants (V777L, D769Y, G776C, and P780 insertion) characterized as hyperactivated in Dr. Miller's laboratory. Atomic-level models have been constructed in two activation states: active-like and Src/CDK-like inactive. The models have been refined with short molecular dynamics simulations and pending additional analysis will be used to begin virtual screening to identify potential inhibitor candidates for purchase and subsequent testing for activity in Dr. Miller's laboratory.

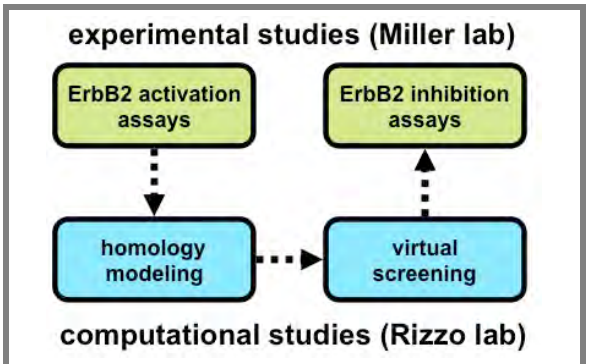

Fig. 1. Interplay between experimental (green) and computational (blue) research teams to identify small molecule ErbB2 drug leads.

Specific Aim 3: Identifying inhibitors of ErbB2 mutants.

In order to construct atomic models of the kinase domain of ErbB2 activating mutants that are suitable for large-scale virtual screening, we first built homology models of wild-type ErbB2 kinase domain. The models were based on crystallographic structures of the kinase domain of ErbB2 and its close relative EGFR (ErbB1). The kinase domains of ErbB2 and EGFR are highly homologous as indicated by a sequence identity of ~ 78%. There are two currently available crystallographic structures of the ErbB2 kinase domain. One is in the "active-like" conformation (Aertgeerts et al, *J Biol Chem*, 2011, 286, 18657-18765) and the other is in the Src/CDK-like "inactive" conformation (Ishikawa et al, *J Med Chem*, 2011, 54, 8030-8050). We completed the coordinates of ErbB2 with corresponding residues from EGFR coordinates and refined the wild-type models (in both activation states, active-like and CDK/Src-inactive) with properly restrained molecular dynamic (MD) simulations.

Four somatic mutants (V777L, D769Y, P780insertion and G776C) of the ErbB2 kinase domain have been expressed and analyzed by Dr. Miller's lab. They have been shown to have consistently higher tyrosine kinase activity than wild-type ErbB2 (described in the Miller Lab report). We have introduced three of the four mutations (V777L, D769Y, G776C) to the wild-type ErbB2 kinase domain models. Each of the mutant models are in both activation states and have been refined with restrained MD simulations. As an example of a completed model, Figure 2 shows a ribbon diagram for D769Y. Ongoing work involves building and refining a model for the P780insertion mutation which is more complicated given the larger change in amino acid composition compared to the previously derived point mutants. Additionally, unrestrained MD simulations for wild-type and mutant models in both apo and ligand-bound conditions can be used to explore possible mechanisms of ErbB2 activation and inhibition.

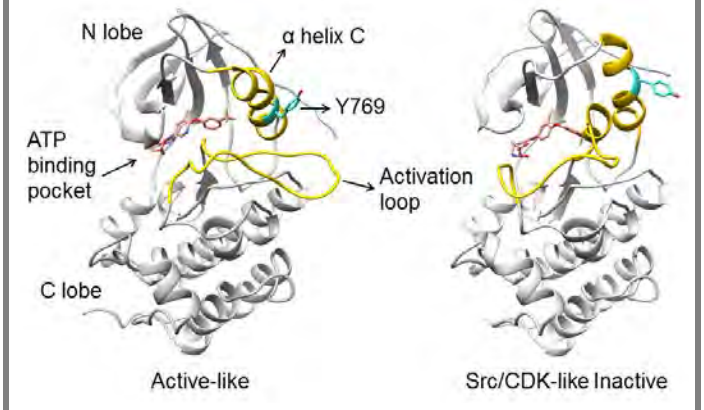

Fig. 2. Atomic models of the ErbB2-D769Y kinase domain in "active-like" (left) and Scr/CDK-like (right) conformations.

In summary, we have achieved Milestone #2 (Production of computationally-derived structures of activated forms of ErbB2), which has prepared us to pursue large-scale virtual screening for promising compounds as originally proposed for Major Task 6.

What opportunities for training and professional development has the project provided?

Progress on Specific Aim 3 has enabled the training for Ms. Jiaye Guo, a graduate student in the Ph.D. program of Biochemistry and Structural Biology at Stony Brook, in homology model construction and refinement of ErbB2 somatic mutants.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

(1) Construct and refine atomic models of the ErbB2 kinase domain mutant (P780insertion) which has been identified with hyperactivity compared to wild-type ErbB2 by Dr. Miller's lab.

(2) Perform virtual screening to the activating mutant models (V777L, D769Y, P780insertion and G776C) of the ErbB2 kinase domain in order to identify and purchase compounds that should preferentially bind to and inhibit each mutant.

(3) Refine and optimize, using additional computational methods including MD simulations and *de novo* design, compounds for which the Miller Lab has confirmed mutant-specific HER2 inhibition.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

The data provided by this project will help enable identification of new small molecule drug-leads that can be used to develop treatments for breast cancer. Specifically, by focusing on the construction and refinement of complete atomic models of wild-type and experimentally confirmed hyper-active HER mutants we are now positioned to continue our studies to characterize how small molecule compounds will interact with these targets at the molecular level. The large-scale virtual screens will employ the refined atomic models to target each mutant individually for which a subset of the mot promising compounds will be purchased and sent to the Miller lab for experimental testing.

Importantly, the complete atomic models derived this reporting period will, in addition to facilitating the virtual screens, are necessary for planned molecular dynamics simulations which will be used to help characterize how the predicted compounds (and eventually those confirmed by experiment) will behave under normal thermal fluctuations. This will enable for time-averaged ensemble properties to be computed including steric packing and electrostatic interaction energies, hydrogen-bonding interactions, geometric stability, and estimates of free energies of binding (potency). Such information will be used to help "refine" the initial hits from the combined virtual/experimental screen.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change.

As noted under "Accomplishments" of the report from Dr. Miller's lab, there was a minor change in the identity of some ErbB2 mutants to be tested.

Actual or anticipated problems or delays and actions or plans to resolve them.

Nothing to report.

Changes that had a significant impact on expenditures.

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

Nothing to report.

Significant changes in use or care of human subjects.

Nothing to report.

Significant changes in use or care of vertebrate animals.

Nothing to report.

Significant changes in use of biohazards and/or select agents.

Nothing to report.

6. PRODUCTS:

Publications, conference papers, and presentations

Journal publications.

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers, and presentations.

2015 Guo, J.; Rizzo, R.C. Protocol development to include solvated molecular footprints in lead discovery. Structural Biology Related to HIV/AIDS-2015 Meeting, Bethesda, MD {Abstract &

Poster}

2015 Guo, J.; Rizzo, R.C. Inhibitor Development Targeting HER2 Incorporating Bridging Water Molecules; 250th American Chemical Society National Meeting & Exposition, Boston, MA {Abstract & Poster (COMP-308)}

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

What individuals have worked on the project?

Name:	Robert C. Rizzo
Project Role:	PI
Researcher Identifier	NIH Commons: RIZZORC
Nearest person month worked:	1
Contribution to Project:	Dr. Rizzo designed experiments, analyzed data, and coordinated efforts with Dr. Miller and colleagues.
Name:	Jiaye Guo
Project Role:	Graduate Student
Researcher Identifier	Stony Brook ID# 108645329
Nearest person month worked:	12
Contribution to Project:	Ms. Guo built and refined ErbB2 models and analyzed data.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS:

Collaborative Award:
This grant agreement is a joint proposal with the following log number and award number:
Log#BC132617
Grant Agreement Number: W81XWH-14-1-0419
Recipient: The Research Foundation of SUNY
Principal Investigator: W. Todd Miller

As outlined in the Contract, Dr. Miller will submit an independent annual report.

9. APPENDICES:

Nothing to report.